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TOPICAL REVIEW

Thyroid iodide efflux: a team effort?

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Abstract The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) play key roles in regulating development, growth and metabolism in pre- and postnatal life. Iodide (I^-) is an essential component of the thyroid hormones and is accumulated avidly by the thyroid gland. The rarity of elemental iodine and I^- in the environment challenges the thyroid to orchestrate a remarkable series of transport processes that ultimately ensure sufficient levels for hormone synthesis. In addition to actively extracting circulating I^-, thyroid follicular epithelial cells must also translocate I^- into a central intrafollicular compartment, where thyroglobulin is iodinated to form the protein precursor to T_4 and T_3. In the last decade, several bodies of evidence render questionable the notion that I^- exits thyrocytes solely via the Cl^-/I^- exchanger Pendrin (SLC26A4), therefore necessitating reconsideration of several other candidate I^- conduits: the Cl^-/H^+ antiporter, CLC-5, the cystic fibrosis transmembrane conductance regulator (CFTR) and the sodium monocarboxylic acid transporter (SMCT1).

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Introduction

More often than not, the ability of the thyroid to accumulate I^- and synthesize hormones essential to life – T_4 and T_3 – is taken for granted. It abruptly invades the consciousness in the wake of disastrous events, for example the failure of the Fukushima Dai-ichi nuclear power plant. At such times, sales of prophylactic potassium iodide tablets spike, as well as concerns about possible contamination of foodstuffs, particularly seafood. These behaviours reflect a basic understanding that all forms of iodine/I^-, including isotopic, not only are taken up avidly, but can be stored for prolonged periods in the thyroid – for better or worse.

In furnishing the key element for synthesis of thyroid hormones, the very mechanisms that fuel such concerns also are critical to pre- and postnatal development of many species, including humans. Thyroid hormone is required for development of practically every system of the body and iodine deficiency disorders are a major cause of growth and cognitive defects in children in under-developed and iodine-poor regions of the world. The fact that simply supplying iodized dietary salt effectively combats these serious consequences of iodine deficiency testifies to the thyroid's efficiency in taking up and utilizing available I^-. In the adult organism, thyroid status governs processes as diverse as metabolism and cardiac function. Its input must be taken into account when considering the

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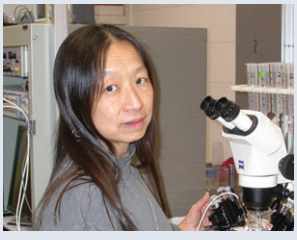

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rapidly expanding literature linking metabolic syndrome and outcomes following cardiac events. It is astonishing that the mechanistic details of one critical cellular process governing thyroid function – I^- accumulation – remain elusive despite the staggering amounts of information amassed. This short review revisits select, pertinent elements of the accumulated knowledge base, with the goal of stimulating future studies directed at understanding the fundamental mechanisms of apical I^- efflux.

The thyroid and production of thyroid hormone

The cellular and tissue organization of the thyroid is critical to understanding the relationship between I^- accumulation and hormone formation. This section provides a brief overview of the key structural and functional features of the thyroid (Carrasco, 1993). Figure 1 illustrates some of the elements underlying thyroid function. Briefly, the thyroid is an epithelial tissue organized as a collection of follicular units that produce, exocytose, iodinate and store thyroglobulin (Tg); these processes subserve hormone production. Tg is an enormous dimeric protein (2×330 kDa monomers) that acts as a scaffold for hormone synthesis by providing specific 'hormonogenic' tyrosine residues. Note that the follicular arrangement facilitates storage of the iodinated Tg, thereby ensuring a supply of hormone precursor. Thyrocytes also take up and process pro-hormone to generate T_4 and T_3, which exit basolaterally into the bloodstream (Fig. 1*B* and *C*). Further details of the iodination, proteolysis and deiodination required for T_4 and T_3 production are elegantly presented in (Kopp *et al.* 2008). All of the described processes are stimulated by the action of thyroid stimulating hormone (TSH, aka thyrotropin) produced by the anterior pituitary. TSH release in turn is subject to feedback regulation by circulating levels of thyroid hormones.

Specific transporters supply the thyroid with I^-, an essential substrate for hormone formation

Thyroid tissue is highly vascularized, a feature that optimizes not only delivery of T_4 and T_3 throughout the body, but also uptake of circulating I^- via cotransport through the sodium iodide symporter, NIS (Dai *et al.* 1996). This secondary active transport process, which is coupled to the Na^+ gradient maintained by the Na^+/K^+-ATPase (Bagchi & Fawcett, 1973), promotes intracellular I^- accumulation to levels higher than in blood. When heterologously expressed in COS-7 cells, human NIS accumulates intracellular I^- to 10-fold the levels achieved by control-transfected cells, whereas levels as high as 40-fold were obtained with expression in HEK-293 cells (Smanik *et al.* 1996; Fujiwara *et al.* 1997). The endogenous Na^+-coupled symport activity in a Fischer rat thyroid line, FRTL-5, raises intracellular I^- to ~30-fold the level in the surrounding medium (Weiss *et al.* 1984). Intracellular I^- levels therefore rise above electrochemical equilibrium. This contributes sufficient driving force for I^- to then traverse the apical membrane and exit into the follicular lumen, where thyroperoxidase (TPO) couples it to specific tyrosine residues within Tg (Fig. 1*C*). Note that this reaction requires H_2O_2, which is furnished by the dual oxidase 2 (DUOX2), a member of the NADPH family (Dupuy *et al.* 1999; De Deken *et al.* 2000; Moreno *et al.* 2002).

Is Pendrin the apical I^- conduit?

Molecular identification of I^- efflux pathway(s) remained elusive until 1997, with the finding by Everett and colleagues that mutations in the *SLC26A4* gene associate with Pendred syndrome, an autosomal recessive disorder characterized by profound sensorineural deafness and occasionally with goitre (Everett *et al.* 1997). The syndrome takes its name from Vaughan Pendred, who first described these symptoms a century earlier (Pendred, 1896). To date, most identified Pendred syndrome mutations are missense mutations (a comprehensive listing can be found at http://www.healthcare.uiowa.edu/labs/pendredandbor/slcMutations.htm). The products of many *SLC26* gene family members function as anion transporters (Mount & Romero, 2004). Subsequent functional studies demonstrated that the product of *SLC26A4*, Pendrin, is capable of mediating I^- transport in a number of heterologous expression systems, including *Xenopus* oocytes as well as mammalian cell systems (Scott *et al.* 1999; Gillam *et al.* 2004). The current working model attributes apical I^- exit to functional Pendrin activity and results from the weight of evidence stemming from clinical observations as well as localization and heterologous expression studies, described further below.

Goitre in pendred syndrome patients and I^- organification defects suggest a role for Pendrin in apical I^- efflux

Pendred syndrome patients sporadically present with euthyroid goitre, generally in the second decade of life. They also may show indications of abnormal I^- organification. In the clinic, I^- organification is evaluated using the perchlorate discharge test. The concept of this assay is depicted in Fig. 2. Subsequent to priming and equilibration of radioactive I^- (e.g. ^{123}I; physical half-life 13.2 h; quantified by gamma emittance over the thyroid), perchlorate is administered. Perchlorate competes with I^- for uptake via NIS (Wolff, 1998;

Tonacchera *et al.* 2004; Tran *et al.* 2008). In unaffected individuals, cellular I^- enters the follicular lumen and is trapped by organification. Thus, on delivery of the competing perchlorate, radioactive emittance changes little despite the now-diminished influx of radioiodide, remaining within 10% of equilibrated levels (Fig. 2*A* and *C*, purple line). In contrast, Pendred patients typically show enhanced release of I^- on challenge with perchlorate, as measured by a marked decline in thyroid gamma emittance. This is referred to as a 'positive perchlorate discharge test' (Fig. 2*B* and *C*, blue line) and indicates impaired organification. In the case of Pendred patients, this implies a reduction in apical I^- transport. Note, however, that the extent of the release can vary widely, and can be used as a means of discriminating partial and total iodide organification defects.

Consistent with the implications of perchlorate discharge tests, cultured Pendred thyroid cells

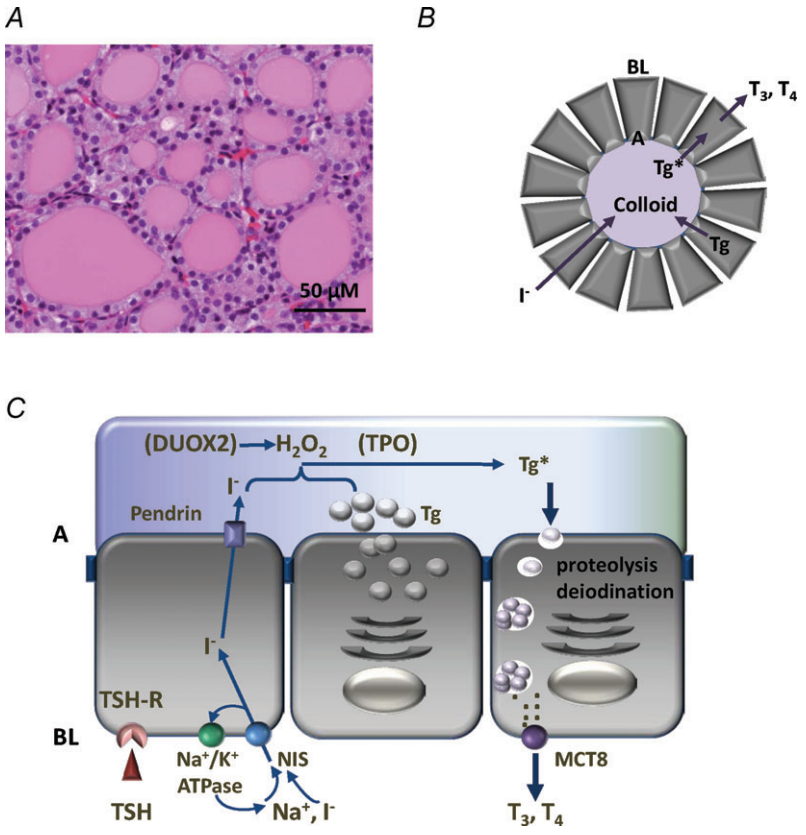

Figure 1. Organization of thyroid follicular epithelium and summary of key processes in hormone synthesis
A, haematoxylin and eosin stained section of neonatal pig thyroid tissue showing follicles consisting of epithelial cells and colloid-filled lumina. Scale bar = 50 μm. *B*, schematic depiction of thyroid follicle showing polarized orientation of epithelial cells (A, apical; BL, basolateral). Arrows indicate directional movements of thyroglobulin (Tg) and I^-, uptake of iodinated Tg (Tg*) pro-hormone and release of T_3 and T_4. Circulating I^- levels in blood range between 10 and 100 μM (Kogai *et al.* 2006; Shcheynikov *et al.* 2008). Due to rapid intrafollicular Tg* formation, the colloid can be regarded as essentially devoid of free I^-. *C*, basic steps involved in thyroid hormone formation are shown. *Left*, TSH binds its receptor and promotes thyroid hormone production. Circulating I^- is taken up at the basolateral membrane via the sodium-iodide symporter, NIS (Dai *et al.* 1996). Intracellular I^- accumulates and moves across the apical membrane by a process believed at least partially to be mediated by Pendrin, thereby providing this essential element for hormone synthesis. *Middle*, Tg is produced by the thyroid epithelial cells, processed along the synthetic pathway and released into the follicular lumen. *Top*, H_2O_2, required for the coupling of I^- and Tg, is generated by DUOX2 at the apical membrane. Reaction of these components is catalysed by TPO; I^- is coupled to many tyrosine residues within Tg, of which only a select number are hormonogenic. Tyrosine residues can be either mono- or di-iodinated. TPO also facilitates coupling of these residues to form precursor to T_3 (mono- + di-iodinated) and T_4 (2 × di-iodinated). *Right*, outline of endocytosis and processing of the pro-hormone. Some Tg* is proteolysed at the apical surface by externalized cathepsins prior to uptake (Brix *et al.* 1996). Internalized Tg* is proteolysed by cathepsins within lysosomes (Dunn *et al.* 1991; Dunn & Dunn, 2001). T_3 and T_4 exit basolaterally in a process at least partially mediated by MCT8, a monocarboxylic acid transporter of the SLC16 family (Di Cosmo *et al.* 2010). Un-utilized iodotyrosines are further degraded by iodotyrosine dehalogenases and recycled.

demonstrate diminished I^- organification as well as T_3 secretion (Sheffield *et al.* 1996). Some Pendred syndrome-affected individuals develop overt hypothyroidism, indicated by elevated serum TSH, with onset during the second decade of life.

Heterologous expression and localization studies provide evidence that Pendrin transports I^-

Pendrin (SLC26A4) is a member of the SLC26A solute carrier family, which comprises transporters having diverse anion exchange functions (Mount & Romero, 2004; Dorwart *et al.* 2008). SLC26A transporters carry an important protein interaction domain, sulfate transporter anti-sigma factor (STAS) (Aravind & Koonin, 2000). The STAS domain has been shown to interact with the regulatory (R) domain of CFTR (Gray, 2004; Ko *et al.* 2004; Dorwart *et al.* 2008; Shcheynikov *et al.* 2008), thereby facilitating inter-molecular cross-talk. In the thyroid, SLC26A4 localizes to the apical membrane of follicular epithelial cells (Royaux *et al.* 2000). Over-expression studies in oocytes indicate that Pendrin transports I^-, Cl^- and formate in exchange for Cl^- (Scott *et al.* 1999; Scott & Karniski, 2000). ^{125}I transport studies performed on MDCK cells co-expressing Pendrin and NIS provide key information from a polarized, mammalian epithelial cell model system (Gillam *et al.* 2004). Monolayers expressing NIS alone accumulated ^{125}I at levels higher than those achieved by monolayers co-expressing Pendrin and NIS. Conversely, medium collected from the apical compartment of co-transfected monolayers contained higher concentrations of ^{125}I than those expressing NIS only. These experimental findings imply that Pendrin can mediate apical I^- efflux. Given its apical localization in thyroid follicular cells, the most direct conclusion followed: Pendrin furnishes I^- for hormone synthesis.

As would be the case, important related questions arise from these findings. Does Pendrin have additional functions that may regulate hormone production? Is Pendrin the only means by which I^- exits into the follicular lumen? What additional players may be involved?

Pendrin also transports HCO_3^-

Several other transporting epithelia express Pendrin, the most notable being the inner ear stria vascularis and the renal collecting duct (Wangemann *et al.* 2004; Wall, 2006). Understanding Pendrin's function in these tissues can elucidate its role in thyroid. In the inner

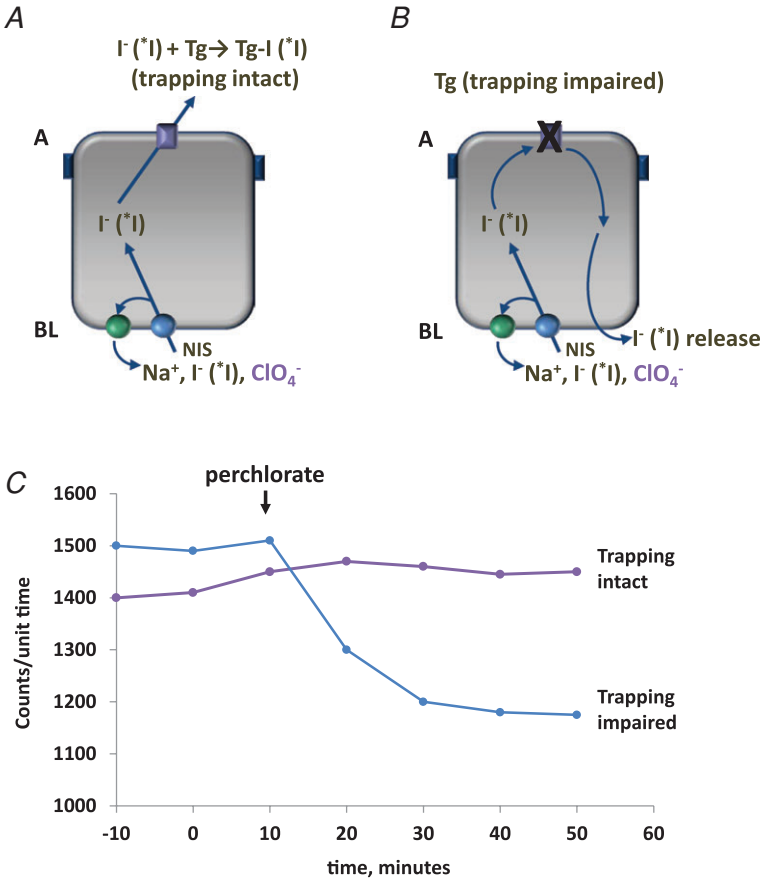

Figure 2. Outline of the perchlorate discharge test
Subjects are infused with radioiodide tracer (*I) prior to perchlorate challenge. *A*, intact trapping depends on efficient exit of I^- (*I) from the cell and into the follicle lumen. On challenge with perchlorate, levels generally remain steady; any decline is <10% below baseline. *B*, impaired exit of I^- (*I) shown in this panel. Trapping cannot proceed without transport of I^- (*I) into the follicular lumen. Perchlorate competes for NIS-mediated uptake; untrapped intracellular I^- (*I) is released basolaterally into the circulation, resulting in a decrease in thyroid counts. *C*, this idealized plot illustrates time courses of radioiodide counts measured over the thyroid after perchlorate administration (arrow) in the case of either intact or impaired trapping.

ear, the lack of HCO_3^- transport by Pendrin disrupts endolymph buffering, launching a sequela of events that result ultimately in deafness (Wangemann, 2006). In the kidney, Pendrin localizes to H^+-ATPase-expressing, intercalated cells of cortical collecting ducts, prompting studies comparing the HCO_3^- secretory capability of alkali-loaded wild-type and Pendrin knockout mice (Royaux *et al.* 2001). Despite metabolic alkalosis, knockout animals failed to secrete HCO_3^-, implying that Pendrin mediates this process. Indeed, one recent clinical case report relates the serious consequences of metabolic alkalosis in Pendred patients (Kandasamy *et al.* 2011). Conversely, in a rat model for metabolic acidosis, Pendrin is down-regulated at both mRNA and protein levels, consistent with a role in mediating HCO_3^- secretion by collecting duct β-intercalated cells (Petrovic *et al.* 2003).

Taken together, these insights underscore the role of Pendrin in both hearing and total animal acid–base homeostasis and moreover identify HCO_3^- as an important substrate. The latter may not be restricted to the cochlea and kidney, as will be discussed.

The plot thickens: *Slc26a4*$^{-/-}$ mice have normal thyroid function

Knockout of *Slc26a4*, the mouse orthologue of *SLC26A4*, has provided a mouse model having both expected and surprising phenotypes. Like Pendred syndrome-affected individuals, *Slc26a4*$^{-/-}$ mice are deaf, as evidenced by the lack of a startle response to a loud clap as well as auditory brainstem recordings (Everett *et al.* 1999). In contrast, no evidence of goitre or hypothyroidism was found in *Slc26a4*$^{-/-}$ mice up to 2 years of age. Wild-type and *Slc26a4*$^{-/-}$ mice did not differ with regard to either animal size or thyroid hormone levels (TSH, total T_3, reverse T_3 and T_4). The lack of phenotype arguably cannot be due to I^--rich lab diets, as dietary I^- restriction failed to induce a thyroid phenotype in *Slc26a4*$^{-/-}$ animals (Calebiro *et al.* 2011). Additional studies systematically showed that the early postnatal surge in T_4 required for key developmental events was intact in *Slc26a4*$^{-/-}$ mice (Wangemann *et al.* 2009). However, intrafollicular pH was more acidic (~0.2 pH units) in knockout animals, suggesting impaired HCO_3^- transport and a role for Slc26a4 in follicular alkalinization. Accordingly, trans-epithelial potential difference was reduced compared to wild-type littermates, indicating an impairment of transport.

Overall, the lack of thyroid phenotype in *Slc26a4*$^{-/-}$ mice suggests that compensatory routes for I^- efflux must participate in thyroid hormonogenesis. If so, can the implications of such findings extend to humans? Moreover, can they account for the variability in thyroid phenotype amongst Pendred syndrome individuals?

Other anion transporters and channels expressed in thyroid: can they mediate I^- efflux?

Thyroid epithelial cells express anion transporters and channels common to many epithelia. Most studies evaluate the ability to carry Cl^- ions, and several will accept I^- as well. There is a substantial electrochemical driving force favouring I^- exit across the apical membrane. The chemical component is maintained by organification, which effectively buffers free I^- in the follicular lumen. The contribution of membrane potential in driving apical I^- efflux is underscored by the dramatic thyroid phenotype of the *KCNE2*$^{-/-}$ mouse (Roepke *et al.* 2009). Deletion of this critical K^+ channel subunit impaired thyroid I^- accumulation and hormone production, and resulted in stunted growth, alopaecia, goitre and other indicators of hypothyroidism. The question arises: can anion transporters and channels previously characterized to transport Cl^- mediate apical I^- efflux in thyroid follicular epithelial cells? The following sections consider three candidates: a Cl^-/H^+ antiporter (CLC-5), sodium-monocarboxylic acid transporter (SMCT1) and the cystic fibrosis transmembrane conductance regulator (CFTR).

CLC-5

CLC-5 is a member of the CLC family of anion transport proteins (Jentsch *et al.* 2005). It is one of the 'intracellular' CLC proteins, residing primarily in endosomes and co-localizing with the H^+-ATPase (Guenther *et al.* 1998). CLC-5 functions as an electrogenic Cl^-/H^+ exchanger, moving two Cl^- ions for every H^+ (Zifarelli & Pusch, 2009). Iodide is transported by CLC-5, albeit less selectively compared to Cl^- (Steinmeyer *et al.* 1995; Mo *et al.* 1999). Mutations in *CLCN5* result in Dent's disease, a condition characterized by renal stones, as well as low molecular weight proteinuria (Lloyd *et al.* 1996). The two different *Clcn5*$^{-/-}$ mouse models recapitulate renal aspects of human Dent's disease to varying extents. Both show low molecular weight proteinuria due to impaired endocytosis by proximal tubules (Piwon *et al.* 2000; Wang *et al.* 2000), whereas calciuria and calcinosis present in only one (Wang *et al.* 2000).

A relationship between CLC-5 and Pendrin expression in the thyroid

CLC-5 is also expressed in thyroid (Maritzen *et al.* 2006; van den Hove *et al.* 2006). Immunoblotting of total thyroid lysates and immunostaining studies indicate reactivity and subapical localization in thyroid sections (van den Hove *et al.* 2006). These investigators further verified the implications of their immunohistochemical analysis by

showing reactivity of anti-CLC-5 with enriched plasma membrane and endosomal membrane samples.

Early studies using both *Clcn5*$^{-/-}$ mouse models tested whether endocytosis or molecules essential for endocytosis, such as megalin, were altered. Neither gave indication of endocytic defects in thyroid. In light of the differences in renal phenotype observed in the two *Clcn5*$^{-/-}$ mouse models, it is not surprising that studies focused on thyroid function also yielded different conclusions. One model showed no apparent disruption of thyroid function (Maritzen *et al.* 2006), whereas several provocative findings emerged in the other (van den Hove *et al.* 2006). Specifically, although *Clcn5* knockout mice were euthyroid on the basis of serum TSH and T_4 measurements, these animals developed goitre at 5 months of age. Autoradiographic evidence, as well as positive perchlorate discharge tests showing 50% release of radioiodide, led to the conclusion that these *Clcn5*$^{-/-}$ mice exhibit a partial I^- organification defect. Interestingly, Pendrin expression was decreased substantially at both RNA and protein levels. Dent's disease patients do not present with goitre; this can be ascribed to the higher rates at which iodinated Tg must be turned over to meet the daily T_4 requirement in mice *vs.* humans (van den Hove *et al.* 2006). It remains unknown whether Pendrin levels are affected in Dent's disease patients.

Can CLC-5 compensate for lack of functional Pendrin?

More recent work from the same group further explores the relationship between Pendrin and CLC-5 (Senou *et al.* 2010). Comprehensive immunohistochemical analysis of thyroid samples from a Pendred syndrome patient carrying the V138F missense mutation revealed a heterogeneous array of changes. Two zones were abnormal, with follicles being either (1) completely destroyed and infiltrated or (2) clearly impaired in the ability to accumulate iodinated Tg. This latter region was characterized by mild CLC-5 expression, but showed increased markers of stress as well as mislocalized DUOX and TPO. A third region that appeared histologically and histochemically normal was analysed further. Subsequent biochemical and PCR analyses of this region revealed substantially upregulated CLC-5 (as well as dehalogenase); all other functional markers were normal. This zone showed normally iodinated Tg, apically localized TPO and DUOX and no indication of oxidative stress or apoptosis. These data suggested that up-regulation of CLC-5 can compensate for loss of Pendrin function in this patient, possibly by directly mediating I^- flux (Fig. 3, left).

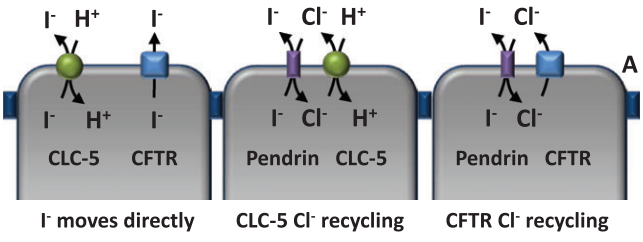

Figure 3. Models showing how CLC-5 and CFTR might directly facilitate I^- efflux, as well as indirectly by coupling with Pendrin
Left, direct I^- transport via either CLC-5 and/or CFTR. *Middle*, efflux of Cl^- through CLC-5 provides a counter-ion for Pendrin-mediated I^- efflux. Note that stoichiometry of CLC-5 is 2 Cl^- to 1 H^+; only one Cl^- is shown for simplicity. *Right*, similarly, CFTR-mediated Cl^- efflux also can furnish a counter-ion for driving Pendrin exchange activity.

Taken together, CLC-5 bears potentially high relevance to thyroid function. Unlike its role in the renal proximal tubule, in the thyroid CLC-5 appears dispensable for endocytic function, yet is important for optimal overall thyroid function. Whether CLC-5 directly transports I^- remains an open question (Fig. 3, left). In its capacity as an electrogenic Cl^-/H^+ antiporter, it could furnish Cl^- as a counter-ion for Cl^-/anion exchange by Pendrin, contribute to a lumen-negative potential difference and increase the luminal pH (Fig. 3, centre). However, the mechanisms underlying its regulatory relationship with Pendrin remain puzzling. How does *Clcn5* knockout result in lower Pendrin levels? Moreover, how does loss of Pendrin function result in upregulation of CLC-5 and what are the mechanisms of compensation? Bridging these gaps in understanding is likely to require integration of multiple experimental approaches.

SMCT1 (hAIT, SLC5A8)

The role of the sodium monocarboxylic acid transporter (SMCT1) in thyroid remains highly controversial. (SMCT1, hAIT and SLC5A8 all refer to the protein product of the *SLC5A8* gene. The term SMCT1 is used generally throughout this discussion; hAIT is used in instances referring to its initial identification.) SMCT1 was originally identified using a RT-PCR-based strategy, with primers targeted at human EST sequences bearing homology to NIS (Rodriguez *et al.* 2002). The assembled sequence contained an open reading frame of 1830 nucleotides, encoding a 610 amino acid protein. An antibody generated against the carboxyl terminus of this protein localized it to the apical pole of human thyroid follicular cells, and hence Rodriguez *et al.* dubbed the protein hAIT (human apical iodide transporter). Iodide transport capability was evaluated by performing ^{125}I uptake experiments subsequent to expression in COS-7 cells, alone or in combination with NIS. Expression alone did not increase accumulation whereas co-expression decreased ^{125}I counts compared to levels achieved by NIS expression alone. These data suggested a role in passive I^- efflux, but did not rule out other transport functions.

SMCT1 is the product of a tumour suppressor gene

Soon thereafter, *SLC5A8* was identified as a putative tumour suppressor gene encoding a protein product identical to hAIT. Its heterologous expression in *Xenopus* oocytes resulted in intracellular Na^+ accumulation (Li *et al.* 2003). SMCT1 expression correlated strongly with survival in colon cancer (Paroder *et al.* 2006). Also demonstrated was a role in Na^+-coupled transport of monocarboxylic acids (Coady *et al.* 2004; Miyauchi *et al.* 2004; Paroder *et al.* 2006); hence the moniker, SMCT1. These findings subsequently led to mechanistic hypotheses linking butyrate uptake with tumour suppressor function in the colon (Thangaraju *et al.* 2006; Singh *et al.* 2010). Early studies in polarized thyroid cultures provided strong evidence for organic anion transport (Gerard *et al.* 1990), nicely complementing SMCT1 expression studies.

SMCT1's physiological substrate in thyroid remains obscure

Several early studies using heterologous systems could not establish I^- transport by SMCT1 (Coady *et al.* 2004; Miyauchi *et al.* 2004; Paroder *et al.* 2006). Moreover, despite disruptions in renal and intestinal lactate transport, the *Slc5a8*$^{-/-}$ mouse lacks a thyroid phenotype; even double knockouts (*Slc5a8*$^{-/-}$/*Slc26a4*$^{-/-}$) showed no changes in thyroid histology or hormone status (Frank *et al.* 2008). These mice did not show increased incidence of tumour formation. It should be noted that these findings apply to adult animals; studies on younger animals may provide further insights, as they have for *Slc26a4*$^{-/-}$ mice (Wangemann *et al.* 2009).

A final word

Before concluding that SMCT1 does not conduct I^-, it must be noted that a more recent study has revisited this issue. Using heterologously expressing *Xenopus* oocytes, I^- currents were measured under conditions of low extracellular Na^+ (Coady *et al.* 2010). Importantly, thyroid epithelia not only secrete anions but also absorb Na^+ avidly (Armstrong *et al.* 1992*a*; Li *et al.* 2010), thereby effectively reducing follicular Na^+. Thus, the jury is still out on the physiological role of SMCT1 in the thyroid.

CFTR

The cystic fibrosis transmembrane conductance regulator gene (*CFTR*) encodes an ATP binding cassette transporter that is found in many secretory epithelia and has diverse functions, the best-studied being its role as a Cl^- channel that is regulated by cAMP-dependent phosphorylation (Hwang & Sheppard, 2009). Disruptions of *CFTR* result in cystic fibrosis (CF) (Riordan *et al.* 1989). Like Pendred syndrome, CF is an autosomal recessive disorder. The most common *CFTR* mutation is a deletion of phenylalanine at position 508 (ΔF508), resulting in protein misfolding. CF disease is associated with a failure to thrive, long attributed to impaired pancreatic enzyme secretion. Even with enzyme therapy, CF-affected individuals tend toward shorter stature and slighter build than unaffected individuals. This suggests that CFTR function also impacts on the generation of hormones important to growth, including thyroid hormone. Pig models for CF recapitulate many aspects of the human disease (Rogers *et al.* 2008; Stoltz *et al.* 2010). Impaired growth of *CFTR*$^{-/-}$ and *CFTR*$^{\Delta F/\Delta F}$ pigs associates with deficiencies in pituitary insulin-like growth factor production (Rogan *et al.* 2010). Developmental anomalies include disrupted airway morphology (Meyerholz *et al.* 2010) that may precipitate the lung infection and disease noted previously in these animals (Rogers *et al.* 2008; Stoltz *et al.* 2010).

Hints of CFTR function in thyroid

Using heterologously expressing Chinese hamster ovary cells, Hanrahan and colleagues showed higher permeability of CFTR to cytoplasmic I^- relative to Cl^- (Tabcharani *et al.* 1992). Thus, if present in thyroid, CFTR could mediate I^- currents. Early studies indicated the presence of cAMP-regulated, serosal to mucosal radioiodide transport, as well as Cl^- secretion, in polarized cultures of thyroid epithelial cells (Nilsson *et al.* 1990; Armstrong *et al.* 1992*a*,*b*). Cell-attached patch recording studies identified a cAMP-activated, linear Cl^- channel in monolayer cultures of pig thyroid epithelial cells. The small single channel conductance (~5.5 pS) and ohmic current–voltage relationship aligned well with CFTR's biophysical profile (Champigny *et al.* 1990). Similar protein kinase A-regulated Cl^- channels in polarized primary thyroid cultures displayed permeability to HCO_3^- as well as Cl^- (Bourke *et al.* 1995). Thus, CFTR-like anion channels have been measured in apical membranes of polarized primary thyroid cells. But are they in fact CFTR?

Is CFTR expressed in thyroid?

This requires confirmation of CFTR expression in thyroid epithelial cells, at least on the molecular level. On the RNA level, CFTR has been detected by RT-PCR in human and pig thyroid (Devuyst *et al.* 1997; Li *et al.* 2010). Presence of CFTR protein in bovine and human thyroid was validated by immunoblot as well as immunocytochemistry (Devuyst *et al.* 1997). Immunoblots of lysates from neonatal *CFTR*$^{+/+}$ and *CFTR*$^{-/-}$ pig thyroid cultures confirmed

expression in $CFTR^{+/+}$ but not $CFTR^{-/-}$ samples (Li *et al.* 2010). Devuyst *et al.* (1997) associated greater numbers of CFTR-positive cells with larger follicles, suggesting a role in follicle growth. In contrast, sections of newborn $CFTR^{+/+}$ and $CFTR^{-/-}$ pig thyroids do not show obvious morphological differences in follicle dimensions (Li *et al.* 2010), suggesting instead that CFTR is dispensable for follicle formation and/or volume maintenance.

cAMP-regulated Cl^- secretion in thyroid requires CFTR, but associated I^- transport must be determined

Functional measurements of polarized primary cultures prepared from neonatal $CFTR^{+/+}$ and $CFTR^{-/-}$ pigs formally confirm the necessity of CFTR expression for cAMP-mediated increases in short-circuit current (Li *et al.* 2010). Specifically, wild-type cultures produced a robust, biphasic Cl^- secretory response to serosal isoproterenol whereas knockout cultures were indifferent to the agonist. Whether CFTR plays a definitive role in I^- efflux awaits systematic analysis. If this is the case, it is imperative to ascertain whether thyroid status ultimately is affected, as well as to distinguish between possible mechanisms. The porcine model will prove critical in such studies. Is I^- efflux directly carried by CFTR under physiological conditions (Fig. 3, left)? Alternatively, is CFTR regulating Pendrin via R and STAS domain interactions, supplying Cl^- for Cl^-/I^- exchange (Fig. 3, right)? Could both mechanisms come into play during different states of thyroid activity?

Additional roles of CFTR in thyroid

If CFTR does not conduct I^- under physiological conditions, its function nonetheless may determine other important aspects of hormonogenesis. For instance, CFTR-mediated Cl^- transport will determine the ionic composition within the follicular lumen, possibly influencing Tg folding, DUOX and TPO activities, and, ultimately, Tg iodination. It also is possible that thyroid epithelial cells, like those lining the airways, require CFTR to engage normal antioxidant defence mechanisms necessary for detoxification of reactive species generated during hormonogenesis (Poncin *et al.* 2008; Trudel *et al.* 2009). If so, CFTR-deficient thyroids may accumulate higher levels of oxidative stress with age.

Conclusion

Approaches as diverse as molecular medicine and single molecule analyses have advanced our understanding of cellular processes involved in thyroid hormone formation and in particular, I^- uptake. These approaches revealed a role of Pendrin in mediating I^-, and possibly HCO_3^-, movement into the follicular lumen. They also continue to provide evidence for the involvement of multiple transport pathways in the overall process of apical I^- exit. Three – CLC-5, SMCT1 and CFTR – have been considered here and more may exist. Studies to date have produced a wealth of information but also indicate that the roles of these transport proteins in I^- accumulation are complex and likely to be inter-related. The challenge now rests not only in unravelling the intricacies of their interactions, but also in weaving these back together in the context of thyroid hormonogenesis. In doing so, testable models from which an integrated picture of their roles in thyroid function can emerge.

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